bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Co-immunoprecipitation of CCNK and DDB1 with 3xFLAG-CDK12 complex

operon cao Xiangbing Qi Ting Han

Updated date: May 31, 2021

An abbreviated version of this protocol was published in eLIFE in Aug 2020

Discovery of a molecular glue promoting CDK12-DDB1 interaction to trigger cyclin K degradation

DOI: 10.7554/eLife.59994

Detailed protocol

Co-immunoprecipitation of CCNK and DDB1 with 3xFLAG-CDK12 complex

1. Couple Anti-FLAG M2 antibody (Sigma-Aldrich, MO, USA F3165) to magnetic epoxy beads (Beijing Yunci Technology Co., Beijing, China) or Dynabeads™ M-270 Epoxy (ThermoFisher 14301) at the ratio of 10 μg of anti-FLAG antibody/mg of beads in the presence of 1 M ammonium acetate and 0.1 M sodium phosphate, pH 7.4 at 37 °C overnight.
2. Scape A549 3xFLAG-*CDK12* knock-in cells from twenty 15-cm plates, wash with DPBS, and then freeze cells in liquid nitrogen.
3. Pulverize frozen cells using a mixer mill MM 400 (Retsch, Haan, Germany) with two rounds of 1-minute ball milling at 30 Hz.
4. Per experiment, solubilize 25 mg of grinded cell powder with 250 μL of IP buffer (50 mM HEPES, pH 7.4, 300 mM NaCl, 0.1% Tween-20) supplemented with 1x cOmplete, Mini, EDTA-free protease inhibitor cocktail (Roche, Bazel, Switzerland).
5. Clarify the resulting lysates by centrifuging at 15,000 g for 10 minutes at 4 °C. Transfer supernatant to a new tube.
6. Supplement clarified lysates with HQ461 or DMSO and incubate at 4°C for 30 minutes.
7. Mix 0.2 mg of anti-FLAG-conjugated magnetic beads with clarified lysates for 15 minutes on a rotating platform at 4 °C.
8. Wash beads with IP buffer supplemented with HQ461 or DMSO for three times.
9. Elute bound proteins from magnetic beads with 1 mg/ml of 3×FLAG peptide (Sigma-Aldrich, MO, USA F4799) with agitation at 4 °C for 30 min. Supplement eluant
10. i-DDB1.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. cao, o. , Qi, X. and Han, T. (2021). Co-immunoprecipitation of CCNK and DDB1 with 3xFLAG-CDK12 complex. Bio-protocol Preprint. bio-protocol.org/prep1122.
2. Lv, L., Chen, P., Cao, L., Li, Y., Zeng, Z., Cui, Y., Wu, Q., Li, J., Wang, J., Dong, M., Qi, X. and Han, T.(2020). Discovery of a molecular glue promoting CDK12-DDB1 interaction to trigger cyclin K degradation. eLIFE. DOI: 10.7554/eLife.59994